Investigation of Hybridization Times for DNA Microarrays

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Large DNA hybridization arrays (microarrays) are used to detect the presence of specific genetic sequences in samples. They are used for many applications including pathogen detection, detection of genetic mutations, and sequencing. Oligos, short strands of single-stranded DNA, are bound to a surface in spots. Complementary DNA strands present in the samples bind (hybridize) to the surface-bound strands and are often optically-labeled to indicate that the binding event has occurred. Detection is determined through correlation of the location of known oligo sequences in the microarray and the indication of a binding event with the DNA in the sample. For a pathogen detection application, for example, the microarray can be populated with oligos with sequences unique to a given pathogen. Positive optical detection of binding implies the presence of the pathogen in the sample.

Microarrays can consist of thousands of different oligos, making microarrays a very powerful tool for multiplexed pathogen detection or sequencing. One drawback of microarrays for pathogen detection is that they can exhibit long hybridization times, up to several hours, making them unattractive for fast detection applications.

Project Goals

The long hybridization time makes the microarrays less attractive for detection when thousands of samples must be processed. Factors affecting the hybridization time were investigated to assess if the process time could be minimized.

Table 1. Typical sample processing steps.

Processing step	Typical processing time
Culture	16 h
Purification	1 h
PCR	1 h
Label	30 min
Hybridize	16 h
Wash	15 min
Read array	15 min

Table 2. Factors affecting hyybridzation times.

Factors affecting hybridization time	Effect	Mitigation steps
Temperature	Activity	Maintain about 40 °C
Concentration of nonbound	Interaction time	Increase concentration, mixing
Number oligos bound	Detection limit	System design
Optics	Detection limit	System design
Background	Detection limit	Wash step
Competitive assay	Interference (2)	Design of assay
Hairpins in oligo	Lower association rate (1)	Design of oligos
Free vs. bound hybridization	Steric effects lowers activity for bound oligos(1)	

Relevance to LLNL Mission

Microarrays are used in the biodetection program at LLNL to identify known pathogens in field samples and ultimately to help identify emerging pathogens through their RNA sequences.

FY2007 Accomplishments and Results

Typical assay processing steps include culturing of the sample, purification of the DNA or RNA, converting RNA to cDNA, performing PCR amplification, labeling the DNA, performing hybridization to the microarray, washing the array and reading the results. The processing time can be lengthy. Typical assay processing times for each step are listed in Table 1.

It is clear from Table 1 that culturing and hybridization times are the main obstacles to obtaining timely assay results. Culturing is used for initial amplification and purification and PCR can be used in some circumstances to substitute for these functions. The elimination of the culturing step will reduce the overall assay time. Several of the factors affecting hybridization time are shown in Table 2. Of the factors shown in Table 2, increased concentration and mixing can have a substantial effect on hybridization times without changing the assay or the hardware. That said, assay/oligo models, including the existence of hairpins in the oligos, which reduce activity, can eliminate gains achieved though increased concentration/mixing.

Microarrays can have tens of thousands of spots per array. The Nimblegen system used by LLNL builds the microarray on a microscope slide over an area measuring approximately 18 mm x 13 mm. The oligo strands arrive at the surface through a combination of convection and diffusion. A typical diffusion coefficient for DNA strands is 1×10^{-7} cm^2/s. Using this number, one can calculate the time required for strands to migrate via diffusion from the edge of a hemisphere of a radius calculated such that the enclosed volume contains the number of strands for a given concentration of the strands in solution. The results of this calculation assuming a 10^6 oligo detection limit is shown in Fig. 1 as the "diffusion" curve. Similar results are plotted for the "Maui" mixer, which is currently used to enhance the mixing of the solution. The Maui mixer is a laminar flow device that pushes the sample fluid back and forth past the chip. While there is convection in the flow direction, vertical and lateral mixing is likely to be the result of diffusion only.

As the concentration of strands in solution decreases, a greater volume of liquid is required to provide the required strands for detection and the reaction time increases to allow the strands to migrate from the limits of the volume to
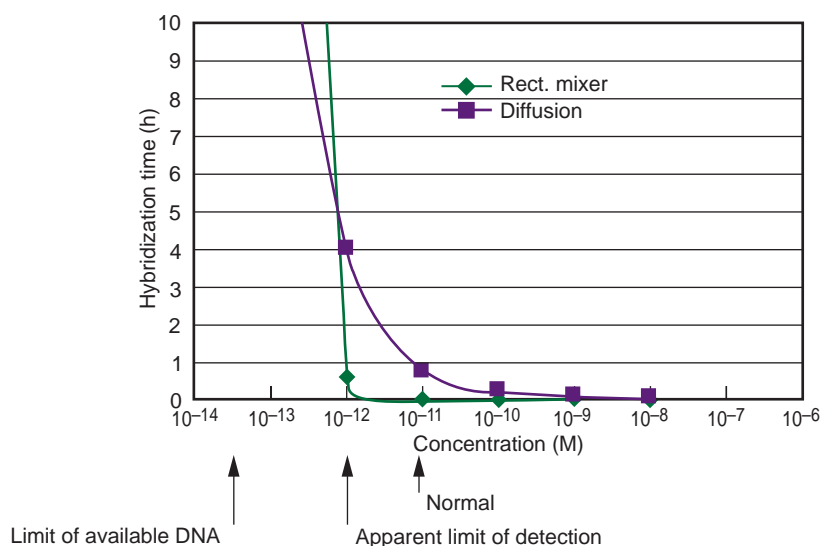
Figure 1. Calculated hybridization times for diffusion (diamonds) and Maui (rectangles) mixer.
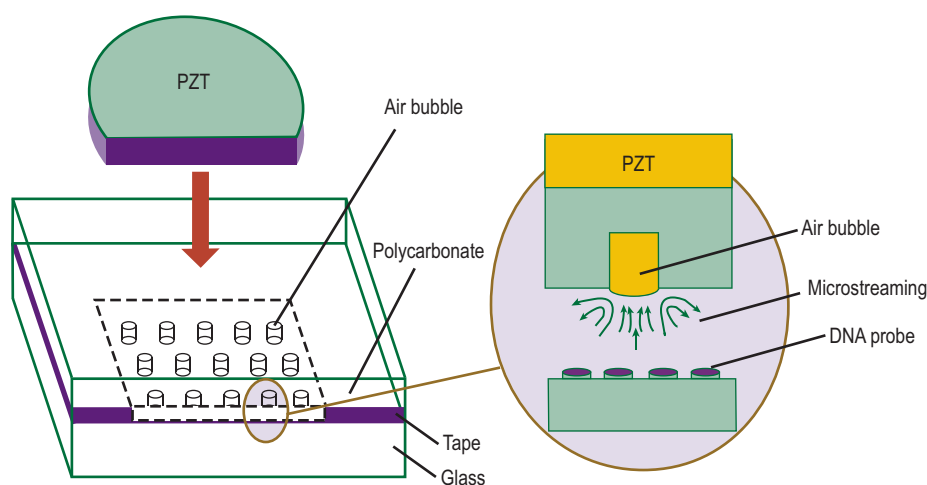

Figure 2. Schematic of acoustic-streaming mixer.

Table 3. Data of hybridization times for various conditions.

DNA Sample Source	Concentration	Mixer Type	Hyb. Time (h)	Detected (Y/N)
E. coli	4.6 x 10^{-11}	Maui	2	Y
E. coli	4.6 x 10^{-11}	Maui	1	Y
E. coli	4.6 x 10^{-11}	Acoustic-streaming	2	Y
E. coli	4.6 x 10^{-11}	Acoustic-streaming	1	Y
E. faecalis	6.5 x 10^{-12}	Maui	2	Y
E. faecalis	2.2 x 10^{-12}	Maui	2	Y – on verge of non-detection

the bound oligos. The knee of the curve is at about 10^{-12} to 10^{-11} M. Above this concentration, reactions happen in less than 1 h. Below this concentration, many hours may be needed to complete the reaction.

Mixing can aid in reducing the hybridization time between the knee and the ultimate limit-of-use, which occurs when the number of oligos in the sample is equal to the detection limit. Improvements over the Maui mixer would consist of a device that provides convective mixing from all parts of the sample volume. On the microscale, acoustic streaming, chaotic advection, and manipulation through electric fields represent methods for effecting mixing in an inherently low-Reynold's number regime. A mixer based on acoustic streaming instigated at the air-water interface of an array of bubbles, as shown in Fig. 2, was built and tested along the Maui mixer. Test data in Table 3 shows that acoustic streaming is at least as effective as the Maui mixer. Future effort will focus on understanding the effects of mixing in both the Maui mixer as well as the acoustic streaming device on the surface hybridization and detection process for various concentrations of the analyte/sample.

Related References

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